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Supplementary Information Titles

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Article Title:	Chemo-enzymatic fluorination of unactivated organic compounds
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Supplementary Item & Number	Title or Caption
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Supplementary Figure 2	Chiral GC traces of purified compound 5 (a) and 7 (b).
Supplementary Figure 3	Chiral GC traces of purified compound 9 (a) and 10 (b).
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Supplementary Figure 5	Chiral GC traces of purified compound 19c (a) and 20c (b).
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Supplementary Table 1	Stereoselective fluorination of substituted 2-aryl acetic acid esters.
Supplementary Table 2	Sequences of the P450 _{BM3} variants.
Supplementary Methods (or Discussion or Data)	Materials, Methods and Synthetic Procedures

Supplementary Information for

Chemo-enzymatic fluorination of unactivated organic compounds

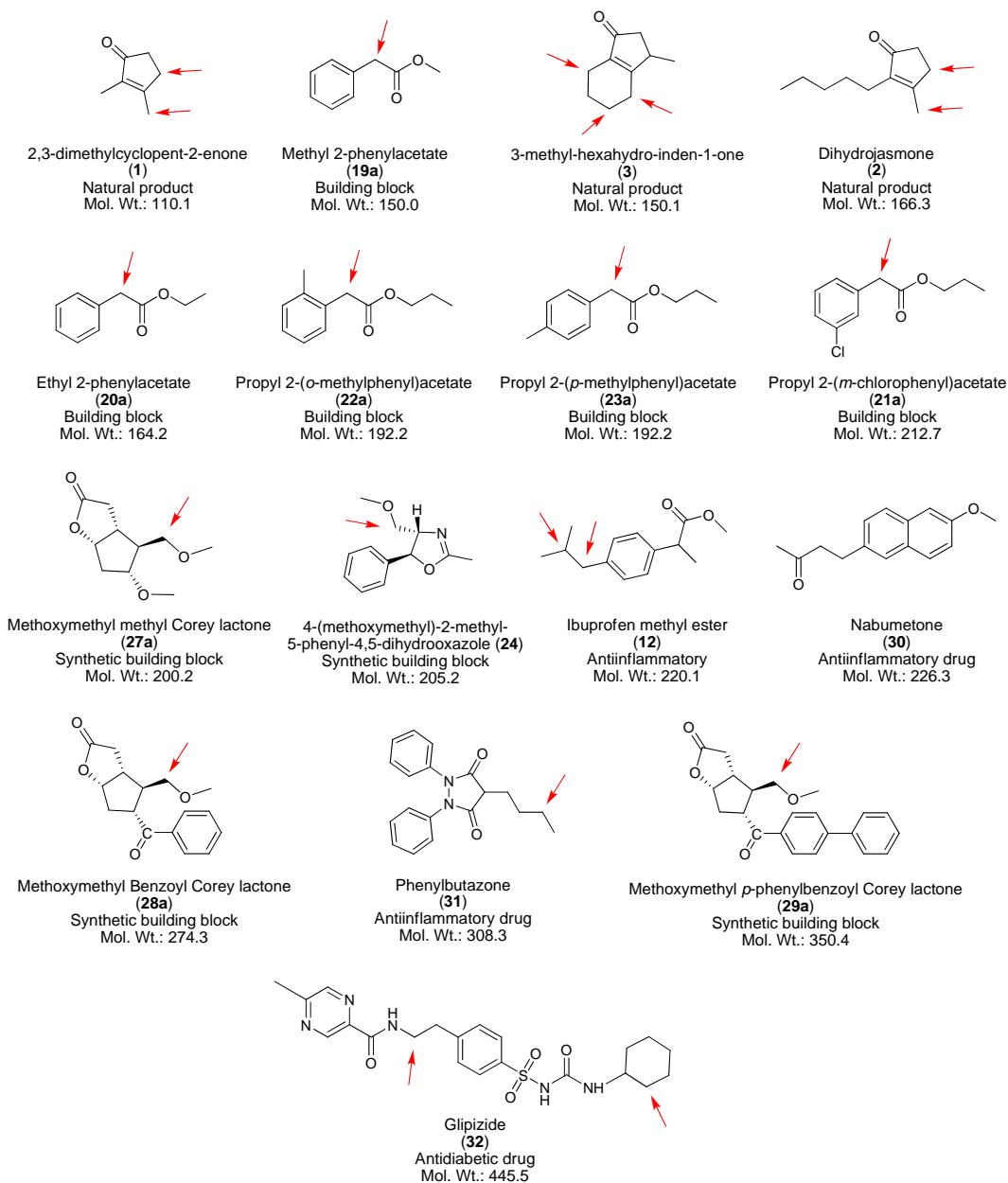
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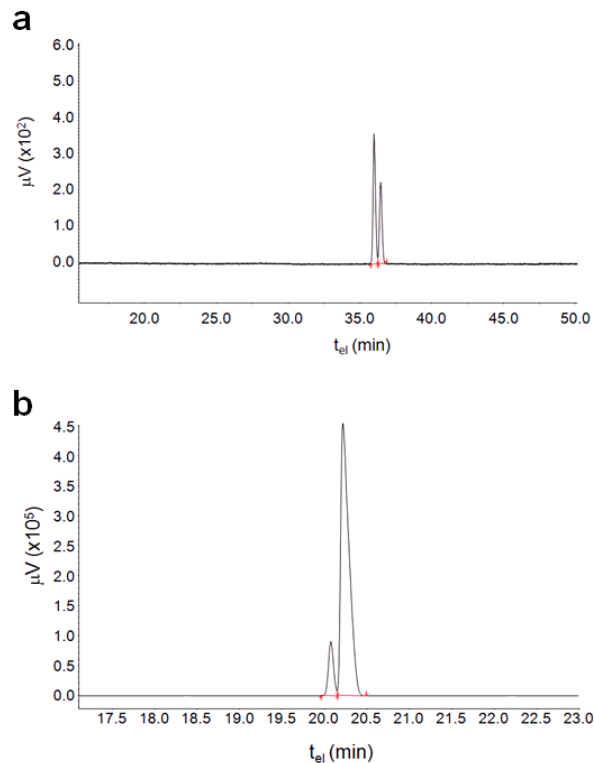
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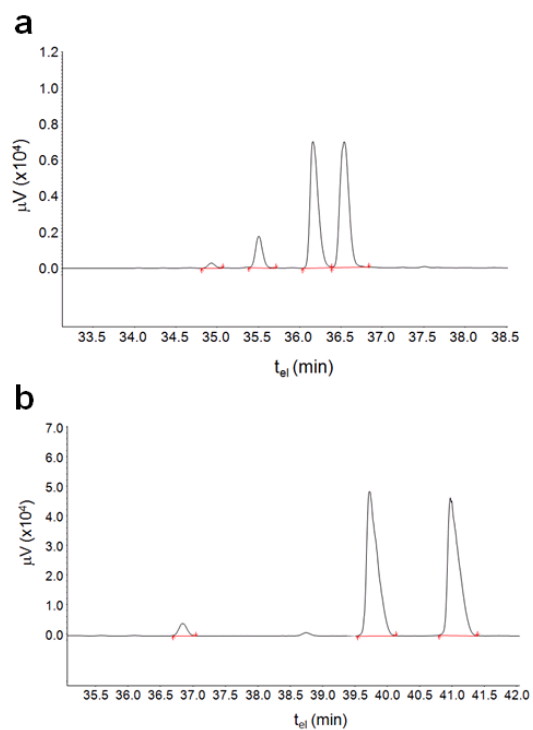
Supplementary Figure 1. Test molecules (ordered according to their molecular weight). Arrows indicate the site(s) targeted by chemo-enzymatic fluorination.



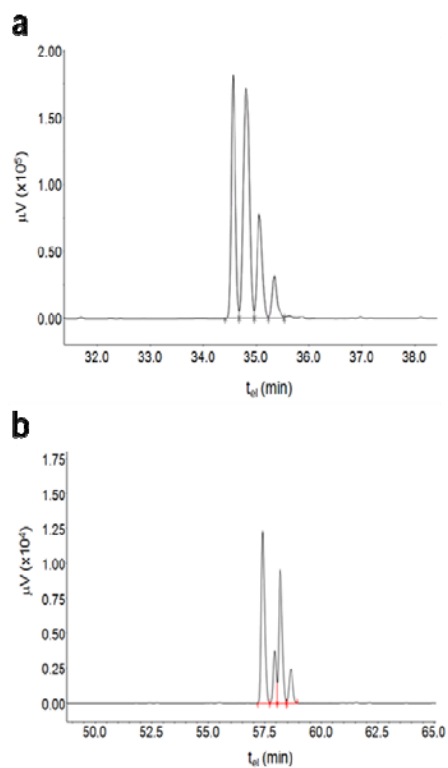
Supplementary Figure 2. Chiral GC traces of purified compound **5** (a) and **7** (b).



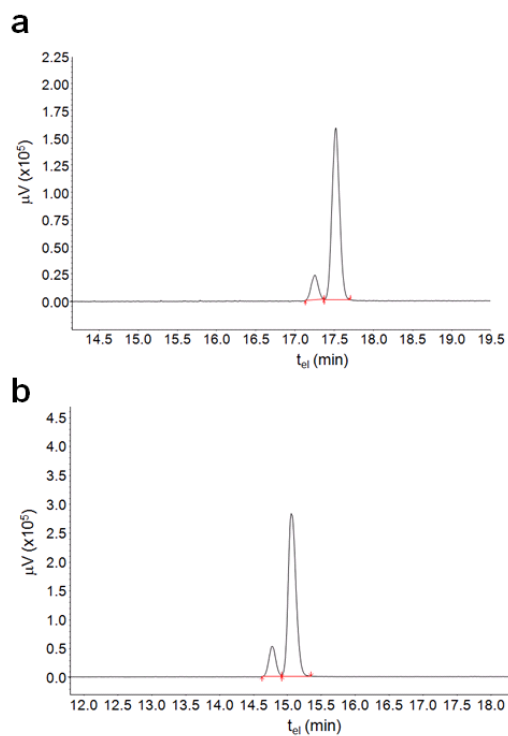
Supplementary Figure 3. Chiral GC traces of purified compound **9** (a) and **10** (b).



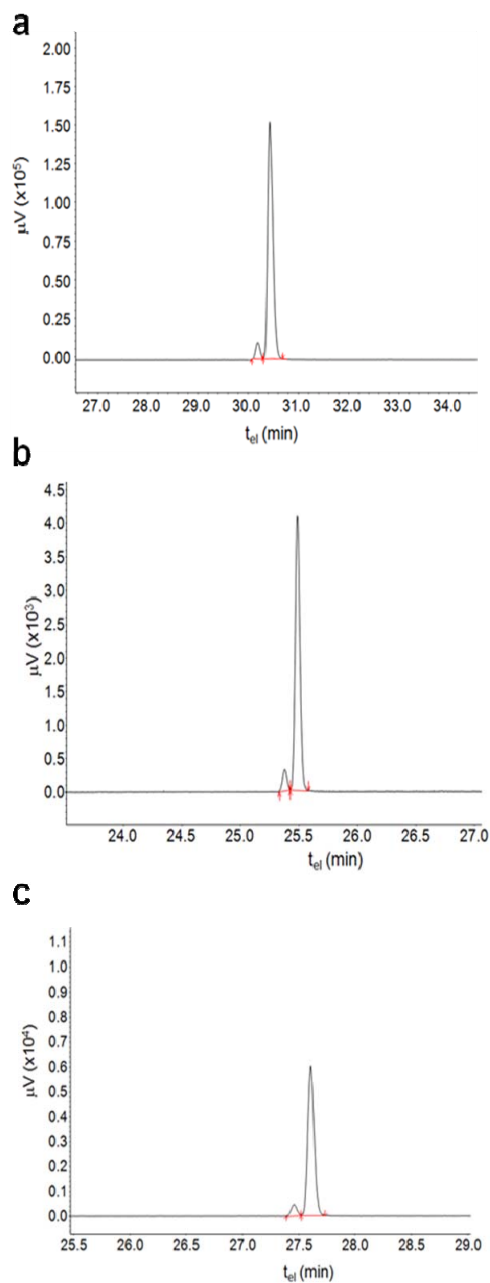
Supplementary Figure 4. Chiral GC traces of purified compound **15** (a) and **18** (b).



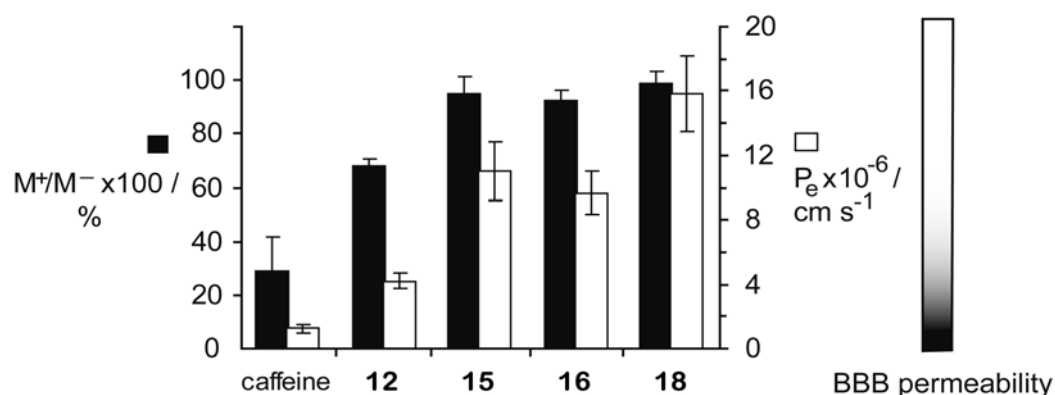
Supplementary Figure 5. Chiral GC traces of purified compound **19c** (a) and **20c** (b).



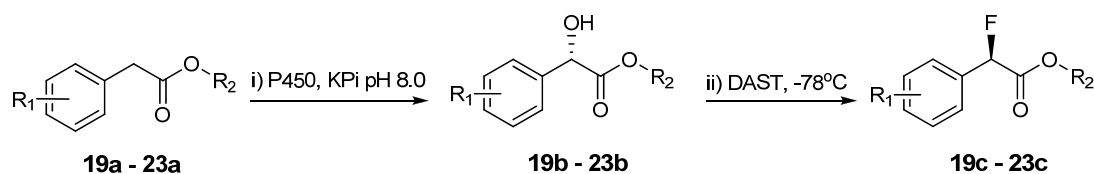
Supplementary Figure 6. Chiral GC traces of purified compound **21c** (a), **22c** (b), and **23c** (c).



Supplementary Figure 7. Membrane permeability properties of **12** and its fluorinated derivatives. Effective permeability values (P_e) are calculated by monitoring accumulation of the organic molecule in a cross-membrane compartment. The correlation between P_e value and BBB-permeability in the absence of active transport has been previously established using a wide set of commercial drugs² and is illustrated by the graded bar (black = not BBB-permeable; white = BBB-permeable). Caffeine served as control for assay validation (literature: $1.30 \times 10^{-6} \text{ cm s}^{-1}$; experimental: $1.24 \times 10^{-6} \text{ cm s}^{-1}$). M^+/M^- refers to the fraction of compound that crossed the BBB-mimic membrane as compared to system with no membrane after 16 hour incubation at room temperature. Values are means of three replicates.



Supplementary Table 1. Stereoselective fluorination of substituted 2-aryl acetic acid esters.



Compound	R ₁	R ₂	P450 ^[a]	mol%	Yield (i) ^[b]	Yield (ii) ^[b]	ee ^[c]
19a	H	Me	var-A3	0.1	45% (19b)	75% (19c)	74%
20a	H	Et	var-B4	0.1	66% (20b)	78% (20c)	72%
21a	<i>m</i> -Cl	Pr	var-C12	0.05	75% (21b)	82% (21c)	89%
22a	<i>o</i> -Me	Pr	var-C12	0.05	65% (22b)	83% (22c)	85%
23a	<i>p</i> -Me	Pr	var-C12	0.05	72% (23b)	88% (23c)	87%

[a] The sequences of the P450 variants are reported in Supplementary Table 2. [b] Yields refer to the isolated product. [c] Enantiomeric excess determined by chiral GC analysis.

Supplementary Table 2. Sequences of the P450_{BM3} variants.

Enzyme	Amino acid mutations compared to wildtype P450 _{BM3}
var-B2 ^[a]	V78A, H138Y, T175I, V178I, A184V, H236Q, E252G, R255S, A290V, A295T, L353V
var-B3 ^[b]	R47C, V78A, K94I, P142S, T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V
var-B4	F87A
var-C12 ^[c]	R47C, V78A, F87A, K94I, P142S, T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V
var-D10 ^[c]	R47C, V78A, A82L, K94I, P142S, T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, A328V, L353V
var-G4 ^[c]	V78A, A82G, F87V, P142S, T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, A328V, L353V
var-G5 ^[c]	V78A, F87I, P142S, T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V
var-G6 ^[c]	V78A, F81P, A82L, F87A, P142S, T175I, A180T, A184V, A197V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V
var-H1 ^[d]	R47C, L52I, V78F, A82S, K94I, P142S, T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, A328L, K349N, L353V, I366V, E464G, I710T
var-H3 ^[a]	V78A, T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V

^[a] var-B2 and var-H3 correspond, respectively, to variants 139-3 and J described in Fasan *et al.*⁴.

^[b] var-B3 corresponds to variant 9-10A described in Meinhold *et al.*⁵.

^[c] These variants are derivatives of variant var-B3.

^[d] var-H1 is a derivative of variant ETS8 described in Fasan *et al.*⁴.

Materials, Methods and Synthetic Procedures

Reagents and Analytical Methods. Chemical reagents, substrates and solvents were purchased from Sigma-Aldrich, Acros Organics, Fluka, and Kaironkem. Silica gel chromatography purifications were carried out using AMD Silica Gel 60 230-400 mesh. Gas chromatography (GC) analyses were carried out using a Shimadzu GC-17A gas chromatograph, a FID detector, and an Agilent HP5 column (30 m x 0.32 mm x 0.1 μ m film). Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, a FID detector, and an Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μ m film). GC-MS analyses were carried out on a Hewlett-Packard 5970B MSD with 5890 GC and a DB-5 capillary column. HPLC analyses were carried out using a Waters 2690 instrument equipped with a 996 Photodiode Array Detector and a Phenomenex Gemini 5u C₁₈ column. HPLC-MS analyses were carried out using an Agilent 1100 Series LC/MSD device and Kromasil 100, 5 μ m C₁₈ column. ¹H, ¹³C, and ¹⁹F NMR spectra were recorded on a Varian Mercury 300 spectrometer (300 MHz, 75 MHz, and 282 MHz, respectively), and are internally referenced to residual solvent peak. Data for ¹H NMR are reported in the conventional form: chemical shift (δ ppm), multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad), coupling constant (Hz), integration). Data for ¹³C and ¹⁹F NMR are reported in the terms of chemical shift (δ ppm) and multiplicity. High-resolution mass spectra were obtained with a JEOL JMS-600H High Resolution Mass Spectrometer at the California Institute of Technology Mass Spectral Facility.

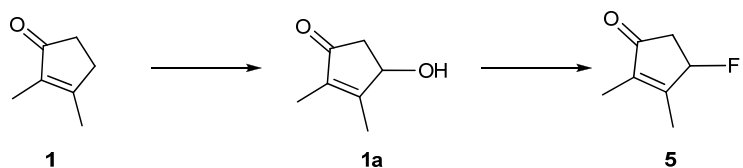
Enzyme Library Screening. Expression of P450_{BM3} variants in 96-well plates and preparation of cell lysates were performed as described.¹ Screening of enzyme activity on cyclopentenone derivatives (**1**, **2**, and **3**) and ibuprofen derivatives (**12** and **16**) was

carried out in 96-well microtiter plates mixing 700 μ L KPi (100 mM, pH 8.0), 200 μ L cell lysate, 10 μ L 200 mM substrate in EtOH, and 100 μ L of a cofactor regeneration solution containing 4 mM NADPH, 100 mM glucose-6-phosphate, and 10 U/mL glucose-6-phosphate dehydrogenase. After incubation for 16 hours at room temperature in orbital shaker, reactions were extracted with chloroform and analyzed by gas chromatography on a Shimadzu GC-17A GC using an Agilent HP5 column (30 m x 0.32 mm x 0.1 μ m film), 1 μ L injection, FID detector, and the following separation method: 300 $^{\circ}$ C inlet, 300 $^{\circ}$ C detector, 70 $^{\circ}$ C oven, 10 $^{\circ}$ C/min gradient to 210 $^{\circ}$ C, 50 $^{\circ}$ C/min gradient to 260 $^{\circ}$ C, and 260 $^{\circ}$ C for 2 min. Screening of enzyme activity on dihydro-4-methoxymethyl-2-methyl-5-phenyl-2-oxazoline (**24**) was carried out on microtiter plates by mixing 100 μ L KPi (100 mM, pH 8.0), 50 μ L cell lysate, 2 μ L 200 mM **24** in DMSO, and 50 μ L KPi 4 mM NADPH. Reaction mixtures were incubated for 30 minutes at room temperature and then added with 50 μ L 150 mM Purpald in 2 M NaOH. Absorbance at 550 nm was recorded after 30 minutes. The most active variants identified in the colorimetric screen were re-tested for selectivity towards demethylation. 1-mL scale reactions were carried out using 1 mM **24**, 0.5 μ M P450, 400 μ M NADPH, 10 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase. After stirring for 16 hours at room temperature, the reaction mixtures were extracted with chloroform and analyzed by GC-MS on a Hewlett-Packard 5970B MSD with 5890 GC using a DB-5 capillary column, 1 μ L injection, 250 $^{\circ}$ C inlet, and the following separation program: 80 $^{\circ}$ C oven, 10 $^{\circ}$ C/min gradient to 250 $^{\circ}$ C, 250 $^{\circ}$ C for 3 min. Enzyme activity on **27a**, **28a**, and **29a** was screened by mixing 50 μ L cell lysate, 100 μ L 100 mM KPi buffer (pH 8.0) containing 1 mM substrate (1% DMSO), and 50 μ L 4 mM NADPH in microtiter plates. After 30 min, 50 μ L 150 mM Purpald (Sigma)

in 2 M NaOH were added to each well. Absorbance at 550 nm was measured after 60 minutes. The most active variants were re-tested at 1-mL scale using 1 mM substrate, 0.5 μ M purified P450, and the cofactor regeneration system described above. After overnight incubation at 4 °C, reactions were analyzed by HPLC-MS using an Agilent 1100 Series LC/MSD device and Kromasil 100, 5 μ m-C₁₈ column.

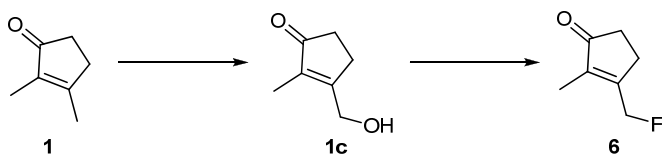
Membrane permeability assay. The membrane permeability assays were carried out adapting a described procedure² and using 96-well MultiScreen-IP plates from Millipore. Filter plates were rinsed with 70% EtOH and double-distilled water prior to use. BBB-mimic membranes were prepared by coating filter wells with 5 μ L porcine brain lipids (PBL, Avanti Polar Lipids) in dodecane (20 mg/mL). Control wells (PBL-free) were primed in the same way but not coated with PBL. Working solutions contained 0.5 mg/mL fluorinated compound in PBS pH 7.4 (5% EtOH). Assembled plates contained 150 μ L working solution in the PBL-coated (or PBL-free) well, 300 μ L PBS (5% EtOH) in the acceptor well, and were incubated at room temperature and 100% humidity. The change in organic compound concentration in the donor and acceptor wells was monitored over time by HPLC. Membrane permeability values (P_e) were calculated as described.³ All measurements were performed in triplicates. The assay was validated by comparing our experimental P_e value for caffeine with that reported by Wohnsland *et al.*³.

Synthetic procedures:



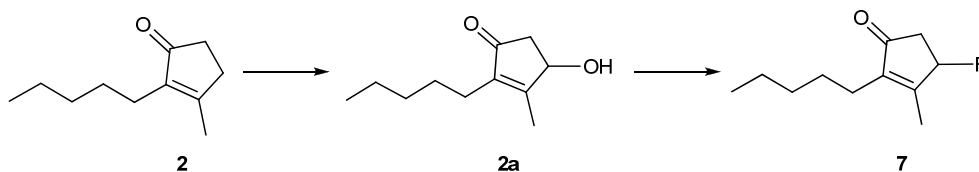
Conversion of 1 to 5. 130 mg (1.18 mmol) **1** were dissolved in 1.5 mL ethanol and added to 200 mL potassium phosphate buffer pH 8.0. The solution was added with var-H3 (final conc.: 2.5 μ M) and a cofactor regeneration system (final conc.: 500 μ M NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 20% ethyl acetate in hexane) to afford **1a** (130 mg, 88%, colorless oil). ^1H -NMR (300 MHz, CDCl_3): δ 1.71 (m, 3H, CH_3), δ 2.08 (m, 3H, CH_3), δ 2.27 (dd, J = 18.3, 1.8 Hz, 1H), δ 2.77 (dd, J = 18.3, 6.3 Hz, 1H), δ 4.73 (m, 1H). ^{13}C -NMR (75 MHz, CDCl_3): δ 8.18, δ 13.91, δ 44.44, δ 71.85, δ 138.37, δ 168.50, δ 205.96. HRMS (EI⁺): exact mass calculated for $\text{C}_7\text{H}_{10}\text{O}_2$ requires m/z 126.0681, found 126.0687. Under argon, 50 mg (0.22 mmol) **1a** and a catalytic amount (3 drops) of ethanol were dissolved in 3 mL dry dichloromethane. The solution was cooled to -78°C and added with 38 μL DAST (0.26 mmol, 1.2 eq) in two aliquots. The reaction was stirred in dry ice for 12 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 10% to 50% dichloromethane in pentane) to afford **5** (25 mg, 90% yield, colorless oil). ^1H -NMR

(300 MHz, CD₂Cl₂): δ 1.68 (dm, J = 4.2 Hz, 3H, CH₃), δ 2.04 (m, 3H, CH₃), δ 2.41 (tm, J = 20.7 Hz, 1H), δ 2.63-2.76 (m, 1H), δ 5.48 (dm, J = 30.6 Hz, 1H). ¹³C-NMR (75 MHz, CD₂Cl₂): δ 7.83, δ 13.40, δ 41.21 (d, J = 19.9 Hz), δ 91.59 (d, J = 173.7 Hz), δ 128.03. ¹⁹F-NMR (282 MHz, CD₂Cl₂): δ -179.4 (dd, J = 51.6, 24.2 Hz). HRMS (EI⁺): exact mass calculated for C₇H₉FO requires m/z 128.0637, found 128.0642. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μ m film) and the following separation program: 300 °C inlet, 300 °C detector, 60 °C oven, 0.5 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An enantiomeric excess of 22% was estimated based on peak areas (see **Supplementary Figure 2**).



Conversion of 1 to 6. 130 mg (1.18 mmol) **1** were dissolved in 1.5 mL ethanol and added to 200 mL potassium phosphate buffer pH 8.0. The solution was added with var-G6 (final conc.: 2.5 μ M) and a cofactor regeneration system (final conc.: 500 μ M NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 50 mL). The combined organic phases were dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 20% ethyl acetate in hexane) to afford **1c** (66 mg, 45%, colorless oil). ¹H-NMR (300 MHz, CDCl₃): δ 1.71 (m, 3H, CH₃), δ 2.4 (dm, J = 4.5 Hz, 2H), δ 2.65 (m, 2H), δ 4.58 (s, 2H). ¹³C-NMR (75 MHz, CDCl₃): δ 8.23, δ 27.18, δ 34.10, δ 61.05, δ 136.15, δ 170.05, δ 210.61. HRMS (EI⁺): exact mass calculated for

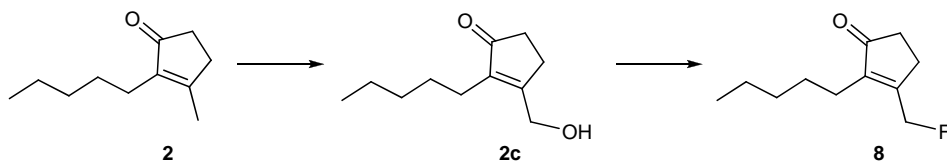
$C_7H_{10}O_2$ requires m/z 126.0681, found 126.0685. Under argon, 50 mg (0.22 mmol) **1c** and a catalytic amount (3 drops) of ethanol were dissolved in 3 mL dry dichloromethane. The solution was cooled to $-78\text{ }^{\circ}\text{C}$ and added with 38 μL DAST (0.26 mmol, 1.2 eq) in two aliquots. The reaction was stirred in dry ice for 12 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 10% to 50% dichloromethane in pentane) to afford **6** (24 mg, 85% yield, colorless oil). ^1H -NMR (300 MHz, CD_2Cl_2): δ 1.67 (m, 3H, CH_3), δ 2.36 (m, 2H), δ 2.56 (m, 2H), δ 5.23 (d, $J = 34.2\text{ Hz}$, 2H). ^{13}C -NMR (75 MHz, CD_2Cl_2): δ 7.95, δ 26.41 (d, $J = 4.3\text{ Hz}$), δ 80.83 (d, $J = 164.0\text{ Hz}$). ^{19}F -NMR (282 MHz, CD_2Cl_2): δ -226.9 (t, $J = 42.3\text{ Hz}$). HRMS (EI $^{+}$): exact mass calculated for $C_7H_9\text{FO}$ requires m/z 128.0637, found 128.0643.



Conversion of 2 to 7. 270 mg (1.62 mmol) **2** were dissolved in 2.5 mL ethanol and added to 250 mL potassium phosphate buffer pH 8.0. The solution was added with var-H3 (final conc.: 3 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 30% ethyl acetate in hexane) to afford **2a** (250 mg, 85%,

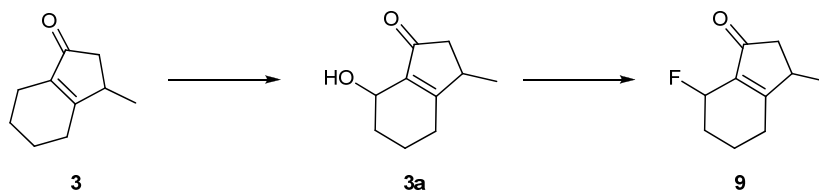
pale yellow oil). ^1H -NMR (300 MHz, CDCl_3): δ 0.87 (t, J = 6.9 Hz, 3H, CH_3), δ 1.25-1.39 (m, 6H), δ 2.08 (s, 3H, CH_3), δ 2.17 (t, J = 8.1 Hz, 2H), δ 2.27 (dd, J = 18.3, 2.1 Hz, 1H), δ 2.77 (dd, J = 18.3, 6.3 Hz, 1H), δ 4.72 (dm, J = 5.1 Hz, 1H). ^{13}C -NMR (75 MHz, CDCl_3): δ 13.83, δ 14.22, δ 22.68, δ 23.16, δ 28.06, δ 28.06, δ 31.99, δ 44.60, δ 71.87, δ 142.73, δ 168.32, δ 205.59. HRMS (EI⁺): exact mass calculated for $\text{C}_{11}\text{H}_{18}\text{O}_2$ requires m/z 182.1307, found 182.1311. Under argon, 200 mg (1.09 mmol) **2a** and a catalytic amount (4 drops) of ethanol were dissolved in 5 mL dry dichloromethane. The solution was cooled to -78°C and added with 200 μL DAST (1.4 mmol, 1.3 eq) in four aliquots. The reaction was stirred in dry ice for 12 hours. The reaction mixture was added with 7 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 20 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 30% ethyl acetate in hexane) to afford **7** (193 mg, 92% yield, pale yellow oil). ^1H -NMR (300 MHz, CDCl_3): δ 0.88 (t, J = 6.6 Hz, 3H, CH_3), δ 1.25-1.40 (m, 6H), δ 2.10 (d, J = 2.1 Hz, 2H, CH_3), δ 2.20 (t, J = 7.1 Hz, 2H), δ 2.51 (tm, J = 22.5 Hz, 1H), δ 2.70-2.82 (m, 1H), δ 5.47 (dd, J = 53.7, 6.0, 1H). ^{13}C -NMR (75 MHz, CDCl_3): δ 13.75, δ 14.21, δ 22.66, δ 23.13, δ 27.90, δ 29.94, δ 31.96, δ 41.46 (d, J = 19.6 Hz), δ 91.42 (d, J = 174.9 Hz), δ 167.62. ^{19}F -NMR (282 MHz, CDCl_3): δ -179.1 (ddd, J = 51.8, 21.4, 9.3 Hz). HRMS (EI⁺): exact mass calculated for $\text{C}_{11}\text{H}_{17}\text{FO}$ requires m/z 184.1263, found 184.1255. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 $^\circ\text{C}$ inlet, 300 $^\circ\text{C}$ detector, 70 $^\circ\text{C}$ oven, 1 $^\circ\text{C}/\text{min}$ gradient to 200 $^\circ\text{C}$, 50 $^\circ\text{C}/\text{min}$ gradient to 250 $^\circ\text{C}$, and 250 $^\circ\text{C}$ for 2 min. An

enantiomeric excess of 78% was estimated based on peak areas (see **Supplementary Figure 2**).



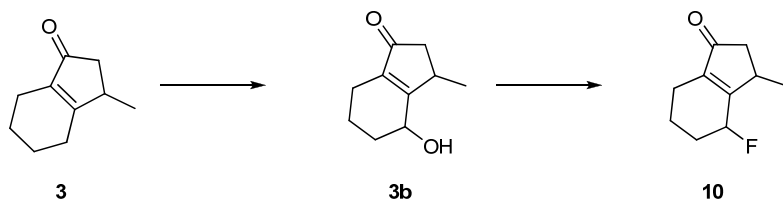
Conversion of 2 to 8. 100 mg (0.60 mmol) **2** were dissolved in 1 mL ethanol and added to 150 mL potassium phosphate buffer pH 8.0. The solution was added with var-G4 (final conc.: 2 μ M) and a cofactor regeneration system (final conc.: 500 μ M NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (3 x 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 30% ethyl acetate in hexane) to afford **2c** (46 mg, 42%, pale yellow oil). GC-MS (EI^+): m/z 182. Under argon, 46 mg (0.25 mmol) **2c** and a catalytic amount (2 drops) of ethanol were dissolved in 2.5 mL dry dichloromethane. The solution was cooled to -78°C and added with 54 μL DAST (0.38 mmol, 1.5 eq) in two aliquots. The reaction was stirred in dry ice for 12 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 30% ethyl acetate in hexane) to afford **8** (62 mg, 89% yield, pale yellow oil). ^1H -NMR (300 MHz, CDCl_3): δ 0.87 (t, J = 6.5 Hz, 3H, CH_3), δ 1.23-1.38 (m, 6H), δ 2.17 (t, J = 7.5 Hz, 2H), δ 2.41 (m, 1H), δ 2.60 (m, 1H), δ 5.26 (d, J = 47.1, 1H). ^{13}C -NMR (75 MHz, CDCl_3): δ 14.32, δ 22.86, δ 23.33, δ 28.46, δ 31.80, δ 31.93, δ 34.87, δ

80.37 (d, $J = 164.8$ Hz). ^{19}F -NMR (282 MHz, CDCl_3): δ -227.9 (t, $J = 48.2$ Hz). HRMS (EI $^+$): exact mass calculated for $\text{C}_{11}\text{H}_{17}\text{FO}$ requires m/z 184.1263, found 184.1263.



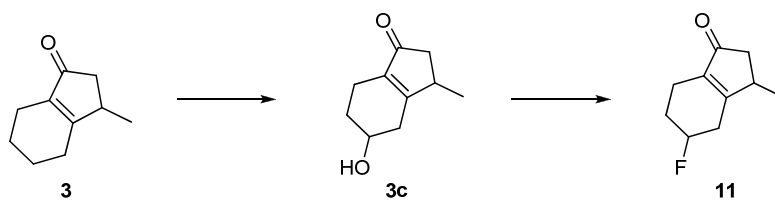
Conversion of 3 to 9. 230 mg (1.53 mmol) **3** were dissolved in 2 mL ethanol and added to 300 mL potassium phosphate buffer pH 8.0. The solution was added with var-D10 (final conc.: 2.5 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 80 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 20% ethyl acetate in hexane) to afford **3a** (175 mg, 69%, colorless oil). GC-MS (EI $^+$): m/z 166. Under argon, 100 mg (0.60 mmol) **3a** and a catalytic amount (3 drops) of ethanol were dissolved in 5 mL dry dichloromethane. The solution was cooled to -78 $^{\circ}\text{C}$ and added with 103 μL DAST (0.72 mmol, 1.2 eq) in two aliquots. The reaction was stirred in dry ice for 3 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 20 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (10% ethyl acetate : 90% hexane) to afford **9** (89 mg, 88% yield, colorless oil). ^1H -NMR (300 MHz, CDCl_3): δ 1.84 (d, $J = 6.6$ Hz), δ 1.45-1.75 (m, 2H), δ 1.80-1.86 (m, 2H), δ 2.03 (m, 1H), δ 2.06 (dd, $J = 18.4, 1.8$ Hz, 1H), δ 2.13-2.21 (m, 1H), δ 2.65 (dd, $J = 18.4, 6.9$ Hz, 1H), δ 2.80 (m, 1H), δ 5.28 (dbr, $J = 50.1$ Hz, 1H). ^{13}C -NMR (75 MHz,

CDCl₃): δ 14.47, δ 18.78, δ 25.89, δ 28.67 (d, $J=21.9$ Hz), δ 36.29, δ 43.89, δ 79.93 (d, $J=164.2$ Hz), δ 135.71 (d, $J=17.9$ Hz), δ 183.76 (d, $J=5.6$ Hz), δ 206.26. ¹⁹F-NMR (282 MHz, CDCl₃): δ -171.4 (m). HRMS (EI⁺): exact mass calculated for C₁₀H₁₃FO requires m/z 168.0950, found 168.0908. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μ m film) and the following separation program: 300 °C inlet, 300 °C detector, 70 °C oven, 1 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. A diastomeric ratio (dr) of 1:8.5, an enantiomeric excess of 5% for the major product, and an enantiomeric excess of 71% for the minor product were estimated based on peak areas (see **Supplementary Figure 3**).



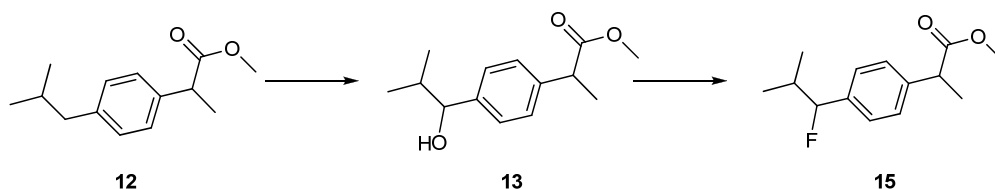
Conversion of 3 to 10. 200 mg (1.33 mmol) **3** were dissolved in 1.5 mL ethanol and added to 200 mL potassium phosphate buffer pH 8.0. The solution was added with var-G4 (final conc.: 3.5 μ M) and a cofactor regeneration system (final conc.: 500 μ M NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 36 hours, the reaction mixture was extracted with dichloromethane (4 x 80 mL). The combined organic phases were dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The residue was purified by flash chromatography (30% ethyl acetate in hexane) to afford **3b** (137 mg, 62%, colorless oil). GC-MS (EI⁺): m/z 166. Under argon, 50 mg (0.30 mmol) **3b** and a catalytic amount (3 drops) of ethanol were dissolved in 3 mL dry dichloromethane. The solution was cooled to -78 °C and added with 51 μ L DAST (0.36 mmol, 1.2 eq) in two aliquots. The

reaction was stirred in dry ice for 5 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (10% ethyl acetate : 90% hexane) to afford **10** (46 mg, 92% yield, colorless oil). ^1H -NMR (300 MHz, CDCl_3): δ 1.27 (d, J = 6.9 Hz, 3H), δ 1.71-1.79 (m, 3H), δ 2.02 (dbr, J = 18.6, 1H), δ 2.03 (br, 1H), δ 2.10-2.13 (m, 1H), δ 2.23-2.35 (m, 1H), δ 2.67 (dd, J =18.6,6.3 Hz, 1H), δ 2.92 (br, 1H), δ 5.20 (dbr, J = 46.2 Hz, 1H). ^{13}C -NMR (75 MHz, CDCl_3): δ 17.55, δ 19.96, δ 20.18 (d, J = 2.8 Hz), δ 29.80 (d, J =21.3 Hz), δ 36.12, δ 44.32, δ 86.48 (d, J = 166.5 Hz), δ 142.79, δ 168.15 (d, J = 13.9 Hz), δ 208.86. ^{19}F -NMR (282 MHz, CDCl_3): δ -174.8 (m). HRMS (EI⁺): exact mass calculated for $\text{C}_{10}\text{H}_{13}\text{FO}$ requires m/z 168.0950, found 168.0913. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 $^\circ\text{C}$ inlet, 300 $^\circ\text{C}$ detector, 70 $^\circ\text{C}$ oven, 1 $^\circ\text{C}/\text{min}$ gradient to 200 $^\circ\text{C}$, 50 $^\circ\text{C}/\text{min}$ gradient to 250 $^\circ\text{C}$, and 250 $^\circ\text{C}$ for 2 min. A diastereomeric ratio (dr) of 4:96 and an enantiomeric excess of 57% for minor product were estimated based on peak areas (see **Supplementary Figure 3**).



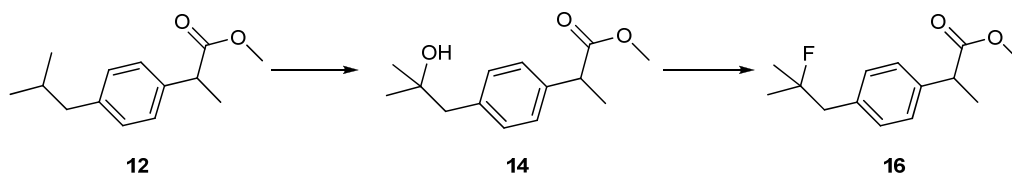
Conversion of 3 to 11. 150 mg (1.0 mmol) **3** were dissolved in 1.5 mL ethanol and added to 200 mL potassium phosphate buffer pH 8.0. The solution was added with var-G5 (final conc.: 3.5 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase)

and stirred at room temperature. After 36 hours, the reaction mixture was extracted with dichloromethane (4 x 80 mL). The combined organic phases were dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The residue was purified by flash chromatography (30% ethyl acetate in hexane) to afford **3c** (53 mg, 32%, colorless oil). GC-MS (EI⁺): m/z 166. Under argon, 53 mg (0.32 mmol) **3c** and a catalytic amount (3 drops) of ethanol were dissolved in 3 mL dry dichloromethane. The solution was cooled to -78 °C and added with 51 µL DAST (0.36 mmol, 1.2 eq) in two aliquots. The reaction was stirred in dry ice for 5 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (10% ethyl acetate : 90% hexane) to afford **11** (48 mg, 90% yield, colorless oil). ¹H-NMR (300 MHz, CDCl₃): δ 1.18 (d, J= 7.2 Hz, 3H), δ 1.45-1.64 (m, 1H), δ 1.79-1.86 (m, 2H), δ 2.00 (dd, J= 18.6, 3.9 Hz, 1H), δ 2.11-2.24 (m, 1H), δ 2.33-2.37 (m, 1H), δ 2.67 (dd, J= 18.6, 6.3 Hz, 1H), δ 2.86 (m, 1H), δ 5.29 (dm, J= 50.1 Hz, 1H). ¹³C-NMR (75 MHz, CDCl₃): δ 17.36, δ 18.62, δ 26.58, δ 28.74 (d, J= 20.7 Hz), δ 37.08, δ 43.67, δ 79.82 (d, J= 164.2 Hz), δ 135.65, δ 208.57. ¹⁹F-NMR (282 MHz, CDCl₃): δ -170.5 (m). HRMS (EI⁺): exact mass calculated for C₁₀H₁₃FO requires m/z 168.0950, found 168.0923. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 µm film). Complete separation of all four diastereomers was not possible.



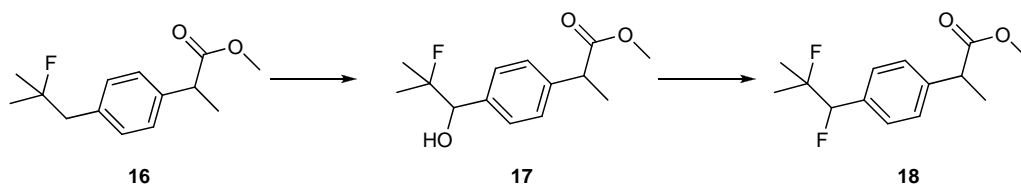
Conversion of 12 to 15. 1.4 mL DMSO containing 150 mg (0.68 mmol) **12** were dissolved in 150 mL 100 mM KPi pH 8.0. The solution was added with var-B4 (final conc.: 4.5 μ M) and a cofactor regeneration system (final conc.: 1 mM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient from 5 to 40% ethyl acetate in hexane) to afford **13** (114 mg, 72%, colorless oil). ^1H -NMR (300 MHz, CDCl_3): δ 0.78 (d, J = 6.9 Hz, 3H, CH_3), δ 0.98 (d, J = 6.9 Hz, 3H, CH_3), δ 1.48 (d, J = 6.9 Hz, 3H, CH_3), δ 1.85-2.00 (m, 1H, CH), δ 3.65 (s, 3H, OCH_3), δ 3.72 (q, J = 6.9 Hz, 1H, CH), δ 4.33 (d, J = 6.9 Hz, 1H, CH), δ 7.25 (m, 4H, CH). ^{13}C -NMR (75 MHz, CDCl_3): δ 18.43, δ 18.81, δ 19.26, δ 35.42, δ 45.32, δ 52.27, δ 79.99, δ 127.09, δ 127.52, δ 139.86, δ 142.77, δ 175.27. HRMS (EI^+): exact mass calculated for $\text{C}_{14}\text{H}_{20}\text{O}_3$ requires m/z 236.1412, found 236.1415. Under argon, 40 mg (0.17 mmol) **13** and a catalytic amount of ethanol were dissolved in 4 mL dry dichloromethane. The solution was cooled to -78°C . After 5 minutes, 29 μL DAST (0.24 mmol, 1.4 eq) were added to the mixture. The reaction was stirred in dry ice overnight. The reaction was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (5% ethyl acetate : 95% hexane) to afford **15** (35 mg, 86% yield,

colorless oil). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 0.84 (d, J = 6.3 Hz, 3H, CH_3), δ 1.01 (d, J = 5.7 Hz, 3H, CH_3), δ 1.48 (d, J = 6.9 Hz, 3H, CH_3), δ 2.05-2.18 (m, 1H, CH), δ 3.66 (s, 3H, OCH_3), δ 3.72 (q, J = 7.5 Hz, 1H, CH), 5.07 (dd, J = 40.0, J = 6.9 Hz, 1H, CHF), δ 7.25 (m, 4H, CH). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): δ 17.78 (d, J = 5.1 Hz), δ 18.58 (d, J = 6.0 Hz), δ 18.81, δ 34.48 (d, J : 85.7 Hz), δ 45.37, δ 52.31, δ 99.3 (d, J = 174 Hz), δ 126.65, δ 126.75, δ 127.56, δ 138.37, δ 140.6, δ 175.16. $^{19}\text{F-NMR}$ (282 MHz, CDCl_3): δ -179.8 (m). HRMS (EI $^+$): exact mass calculated for $\text{C}_{14}\text{H}_{19}\text{FO}_2$ requires m/z 238.1369, found 238.1367. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 $^\circ\text{C}$ inlet, 300 $^\circ\text{C}$ detector, 70 $^\circ\text{C}$ oven, 1 $^\circ\text{C}/\text{min}$ gradient to 200 $^\circ\text{C}$, 50 $^\circ\text{C}/\text{min}$ gradient to 250 $^\circ\text{C}$, and 250 $^\circ\text{C}$ for 2 min. A diastomeric ratio (dr) of 1:3.2, an enantiomeric excess of 19% for the major product, and an enantiomeric excess of 44% for the minor product were estimated based on peak areas (see **Supplementary Figure 4**).



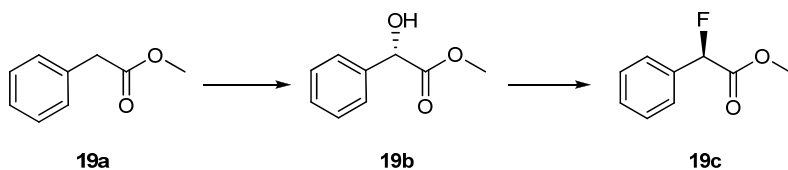
Conversion of 12 to 16. 2.5 mL DMSO containing 220 mg (1 mmol) **12** were dissolved in 250 mL 100 mM KPi pH 8.0. The solution was added with var-G4 (final conc.: 2 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 80 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and

evaporated under reduced pressure. The residue was purified by flash chromatography (gradient from 5 to 40% ethyl acetate in hexane) to afford **14** (208 mg, 88%, colorless oil). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 1.21 (s, 6H, CH_3), δ 1.48 (d, J = 7.2 Hz, 3H, CH_3), δ 2.73 (s, 2H, CH), δ 3.65 (s, 3H, OCH_3), δ 3.70 (q, J = 7.2 Hz, 1H, CH), δ 7.15 (d, J = 8.1, 2H, CH), δ 7.23 (d, J = 8.1, 2H, CH). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): δ 18.84, δ 29.41 (2C), δ 45.26, δ 49.51, δ 52.29, δ 71.00, δ 127.53 (2C), δ 130.98 (2C), δ 136.82, δ 138.92, δ 175.33. HRMS (EI^+): exact mass calculated for $\text{C}_{14}\text{H}_{20}\text{O}_3$ requires m/z 236.1412, found 236.1413. Under argon, 120 mg (0.51 mmol) **14** and a catalytic amount of ethanol (3 drops) were dissolved in 6 mL dry dichloromethane. The solution was cooled to -78°C . After 5 minutes, 87 μL DAST (0.61 mmol, 1.4 eq) were added to the mixture in three aliquots. The reaction was stirred in dry ice overnight. The reaction was then added with 8 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 20 mL). The organic phase was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (5% ethyl acetate: 95% hexane) to afford **16** (114 mg, 95% yield, colorless oil). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 1.32 (d, J = 23.6 Hz, 6H, CH_3), δ 1.48 (d, J = 6.9 Hz, 3H, CH_3), δ 2.87 (d, J : 20.4 Hz, 2H), δ 3.65 (s, 3H, OCH_3), δ 3.70 (q, J = 7.1 Hz, 1H), δ 7.15 (d, J = 8.1 Hz, 2H, CH), δ 7.21 (d, J = 8.1 Hz, 2H, CH). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): δ 18.80, δ 26.83 (d, J : 24.2 Hz), δ 45.24, δ 47.37 (d, J : 22.8 Hz), δ 52.25, δ 98.37 (d, J = 264 Hz), δ 129.12 (d, J : 258.8 Hz), δ 127.39, δ 130.85, δ 136.07, δ 138.92, δ 175.31. $^{19}\text{F-NMR}$ (282 MHz, CDCl_3): δ -137.7 (m). HRMS (EI^+): exact mass calculated for $\text{C}_{14}\text{H}_{19}\text{FO}_2$ requires m/z 238.1369, found 238.1370.



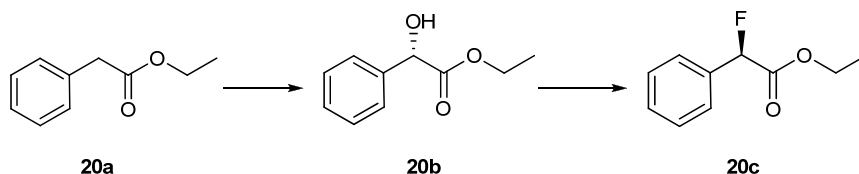
Conversion of 16 to 18. 2 mL DMSO containing 80 mg (0.33 mmol) **16** were dissolved in 200 mL 100 mM KPi pH 8.0. The solution was added with var-B2 (final conc.: 1 μ M) and a cofactor regeneration system (final conc.: 500 μ M NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 80 mL). The combined organic phases were dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The residue was purified by flash chromatography (5% ethyl acetate: 95% hexane) to afford **17** (78 mg, 93%, colorless oil). ¹H-NMR (300 MHz, CDCl₃): δ 1.31 (d, J= 22.5 Hz, 3H, CH₃), δ 1.32 (d, J= 22.5 Hz, 3H, CH₃), δ 1.48 (d, J= 7.2 Hz, 3H, CH₃), δ 3.65 (s, 3H, OCH₃), δ 3.72 (q, J= 7.2 Hz, 1H, CH), δ 4.69 (d, J= 11.7 Hz, 1H, CH), δ 7.26 (d, J= 8.1 Hz, 2H, CH), δ 7.34 (d, J= 8.1 Hz, 2H, CH). ¹³C-NMR (75 MHz, CDCl₃): δ 18.79, δ 21.55 (d, J= 23.1 Hz), δ 24.08 (d, J= 23.7 Hz), δ 45.31, δ 52.28, δ 79.29 (d, J= 23.6 Hz), δ 98.14 (d, J= 168 Hz), δ 127.42, δ 127.91, δ 138.28 (d, J= 5.7 Hz), δ 140.55, δ 175.12. ¹⁹F-NMR (282 MHz, CDCl₃): δ -146.8 (m). HRMS (EI⁺): exact mass calculated for C₁₄H₂₀FO₃ requires m/z 255.1396, found 255.1394. Under argon, 30 mg (0.12 mmol) **17** and a catalytic amount of ethanol were dissolved in 3 mL dry dichloromethane. The solution was cooled to -78 °C. After 5 minutes, 20 μ L DAST (0.14 mmol, 1.2 eq) were added to the mixture. The reaction was stirred in dry ice overnight. The reaction was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The residue

was purified by flash chromatography (5% ethyl acetate: 95% hexane) to afford **18** (30 mg, 98% yield, colorless oil). ^1H -NMR (300 MHz, CDCl_3): δ 1.32 (m, 6H, CH_3), δ 1.51 (d, J = 7.2 Hz, 3H, CH_3), δ 3.66 (s, 3H, OCH_3), δ 3.74 (q, J = 7.2 Hz, 1H, CH), δ 5.29 (dd, J = 45.0, 13.8 Hz, 1H, CHF), δ 7.31 (s, 4H, CH). ^{13}C -NMR (75 MHz, CDCl_3): δ 18.80, δ 23.01 (m), δ 45.38, δ 52.34, δ 96.80 (d, J = 179 Hz), δ 96.52 (d, J = 179 Hz), δ 127.50, δ 127.59, δ 134.8 (d, J = 22.5), δ 141.28, δ 175.03. ^{19}F -NMR (282 MHz, CDCl_3): δ -150.7 (m), δ -187.8 (dd, J = 45.6, 9.0 Hz). HRMS (EI^+): exact mass calculated for $\text{C}_{14}\text{H}_{19}\text{F}_2\text{O}_2$ requires m/z 257.1353, found 257.1356. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 $^\circ\text{C}$ inlet, 300 $^\circ\text{C}$ detector, 70 $^\circ\text{C}$ oven, 0.5 $^\circ\text{C}/\text{min}$ gradient to 200 $^\circ\text{C}$, 50 $^\circ\text{C}/\text{min}$ gradient to 250 $^\circ\text{C}$, and 250 $^\circ\text{C}$ for 2 min. A diastomeric ratio (dr) of 1:3.7, an enantiomeric excess of 9% for the major product, and an enantiomeric excess of 9% for the minor product were estimated based on peak areas (see **Supplementary Figure 4**).



Conversion of 19a to 19c. 90 mg (0.6 mmol) **19a** were dissolved in 500 μL ethanol and added to 240 mL 100 mM KPi buffer pH 8.0. Var-A3 was added to the mixture at a final concentration of 2 μM . The mixture was added with a cofactor regeneration solution containing 0.5 mM NADPH, 30 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase (final concentrations). The reaction mixtures were stirred at room temperature and extracted with chloroform (3 x 100 mL) after 4 hours. The organic phase was then dried over anhydrous MgSO_4 and evaporated under reduced

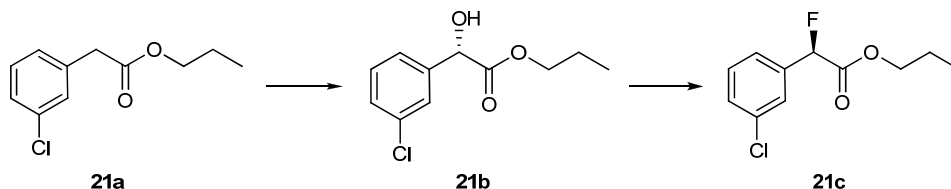
pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **19b** (45 mg). 45 mg (0.27 mmol) of **19b** and a catalytic amount (3 drops) of ethanol were dissolved in 2 mL dry dichloromethane under argon. The solution was cooled to -78 °C and added with 43 μ L DAST (0.30 mmol, 1.2 eq). The reaction was stirred in dry ice for 12 hours. The reaction mixture was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were then dried over anhydrous MgSO_4 and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **19c** (34 mg, 75% yield, pale yellow oil) in 74% *ee*, as determined by chiral GC analysis. ^1H -NMR (300 MHz, CDCl_3): δ 3.75 (s, 3H), δ 5.77 (d, J = 48 Hz, 1H, -CHF), δ 7.37-7.46 (m, 5H); ^{13}C -NMR (75 MHz, CDCl_3): δ 52.8, δ 89.5 (d, J = 184.5 Hz), δ 126.8, δ 126.9, δ 129.0, δ 129.9, δ 134.4 (d, J = 34.5 Hz), δ 169.0. ^{19}F -NMR (282 MHz, CDCl_3): δ -180.29 (d, J = 48.7 Hz). HRMS (EI+): exact mass calculated for $\text{C}_9\text{H}_9\text{FO}_2$ requires m/z 168.0587, found 168.0594. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 °C inlet, 300 °C detector, 100 °C oven, 2 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An enantiomeric excess of 74% was estimated based on peak areas (see **Supplementary Figure 5**).



Conversion of 20a to 20c. 100 mg (0.61 mmol) **20a** were dissolved in 500 μL ethanol and added to 250 mL 100 mM KPi buffer pH 8.0. var-B4 was added to the mixture at a

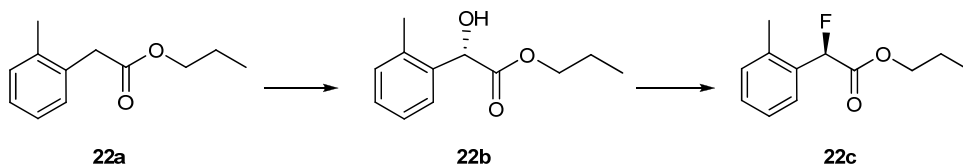
final concentration of 2 μ M. The mixture was added with a cofactor regeneration solution containing 0.5 mM NADPH, 30 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase (final concentrations). The reaction mixtures were stirred at room temperature and extracted with chloroform (3 x 100 mL) after 3 hours. The organic phases were collected, dried over anhydrous MgSO_4 , and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **20b** (72 mg, 66%). 72 mg (0.4 mmol) of **20b** and a catalytic amount (3 drops) of ethanol were dissolved in 2 mL dry dichloromethane under argon. The solution was cooled to $-78\text{ }^{\circ}\text{C}$ and added with 61 μ L DAST (0.43 mmol, 1.2 eq). The reaction was stirred in dry ice for 12 hours. The reaction mixture was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous MgSO_4 and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **20c** (57 mg, 78% yield, pale yellow oil) in 72% *ee*, as determined by chiral GC analysis. ^1H -NMR (300 MHz, CDCl_3): δ 1.24 (t, J = 7.2 Hz, 3H, $-\text{CH}_3$), δ 4.16-4.27 (m, 2H, $-\text{OCH}_2$), δ 5.75 (d, J = 48 Hz, 1H, $-\text{CHF}$), δ 7.37-7.46 (m, 5H); ^{13}C -NMR (75 MHz, CDCl_3): δ 14.2, δ 62.0, δ 81.2, δ 89.6 (d, J = 184.5 Hz), δ 126.8, δ 126.9, δ 128.9, δ 129.8, δ 134.4 (d, J = 34.5 Hz), δ 169.0. ^{19}F -NMR (282 MHz, CDCl_3): δ -180.27 (d, J = 48.7 Hz). HRMS (EI⁺): exact mass calculated for $\text{C}_{10}\text{H}_{11}\text{FO}_2$ requires m/z 182.0743, found 182.0750. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μ m film) and the following separation program: 300 $^{\circ}\text{C}$ inlet, 300 $^{\circ}\text{C}$ detector, 100 $^{\circ}\text{C}$ oven, 2 $^{\circ}\text{C}/\text{min}$ gradient to

200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An enantiomeric excess of 72% was estimated based on peak areas (see **Supplementary Figure 5**).



Conversion of 21a to 21c. 95 mg (0.45 mmol) **21a** were dissolved in 500 μ L ethanol and added to 250 mL 100mM KPi buffer pH 8.0. Var-C12 was added to the mixture at a final concentration of 1 μ M. The mixture was added with a cofactor regeneration solution containing 0.5 mM NADPH, 30 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase (final concentrations). The reaction mixtures were stirred at room temperature and extracted with chloroform (3 x 100 mL) after 4 hours. The organic phases were collected, dried over anhydrous MgSO_4 , and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **21b** (71 mg, 75%). 70 mg (0.34 mmol) of **21b** and a catalytic amount (3 drops) of ethanol were dissolved in 2 mL dry dichloromethane under argon. The solution was cooled to -78 °C and added with 64 μ L DAST (0.45 mmol, 1.5 eq). The reaction was stirred in dry ice for 12 hours. The reaction mixture was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous MgSO_4 , and evaporated under reduced pressure. Purification of the resulting oil by flash chromatography (5% ethyl acetate : 95% hexane) afforded **21c** (63 mg, 82% yield, colorless oil) in 89% *ee*, as determined by chiral GC analysis. ^1H -NMR (300 MHz, CDCl_3): δ 0.85 (t, J = 7 Hz, 3H, $-\text{CH}_3$), δ 1.56-1.68 (m, 2H, CH_2), δ 4.12 (t, J = 6 Hz, 2H,

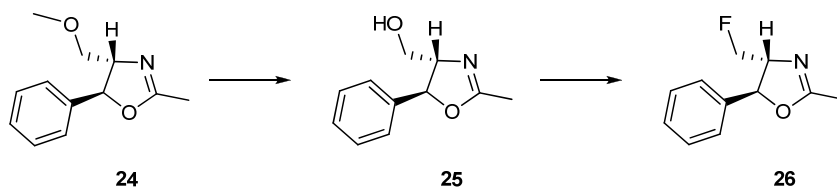
-OCH₂), δ 5.72 (d, J = 48 Hz, 1H, -CHF), δ 7.32 (br, 3H), δ 7.44 (br, 1H). ¹³C-NMR (75 MHz, CDCl₃): δ 10.3, δ 21.9, δ 67.7, δ 88.7 (d, J = 186.5 Hz), δ 124.8, δ 126.9, δ 129.9, δ 130.3, δ 134.9. ¹⁹F-NMR (282 MHz, CDCl₃): δ -182.8 (d, J = 48.7 Hz). HRMS (EI+): exact mass calculated for C₁₁H₁₂ClFO₂ requires m/z 230.0510, found 230.0502. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μ m film) and the following separation program: 300 °C inlet, 300 °C detector, 100 °C oven, 2 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An enantiomeric excess of 89% was estimated based on peak areas (see **Supplementary Figure 6**).



Conversion of 22a to 22c. 100 mg (0.52 mmol) of **22a** were dissolved in 500 μ L ethanol and added to 250 mL 100 mM KPi buffer pH 8.0. Var-C12 was added to the mixture at a final concentration of 1 μ M. The mixture was added with a cofactor regeneration solution containing 0.5 mM NADPH, 30 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase (final concentrations). The reaction mixtures were stirred at room temperature and extracted with chloroform (3 x 100 mL) after 4 hours. The organic phases were collected, dried over anhydrous MgSO₄, and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **22b** (78 mg, 65%). 78 mg (0.37 mmol) of **22b** and a catalytic amount (3 drops) of ethanol were dissolved in 4 mL dry dichloromethane

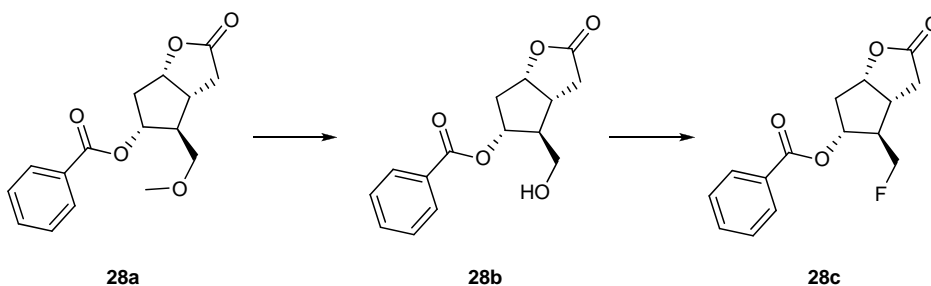
mixture at a final concentration of 1 μ M. The mixture was added with a cofactor regeneration solution containing 0.5 mM NADPH, 30 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase (final concentrations). The reaction mixtures were stirred at room temperature and extracted with chloroform (3 x 100 mL) after 4 hours. The organic phases were collected, dried over anhydrous MgSO_4 , and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **23b** (70 mg, 65%). 70 mg (0.34 mmol) of **23b** and a catalytic amount (3 drops) of ethanol were dissolved in 4 mL dry dichloromethane under argon. The solution was cooled to $-78\text{ }^\circ\text{C}$ and added with 58 μ L DAST (0.4 mmol, 1.5 eq). The reaction was stirred in dry ice for 12 hours. The reaction mixture was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous MgSO_4 , and evaporated under reduced pressure. Purification of the resulting oil by flash chromatography (5% ethyl acetate : 95% hexane) afforded **23c** (59 mg, 83%, colorless oil) in 87% ee, as determined by chiral GC analysis. ^1H -NMR (300 MHz, CDCl_3): δ 0.84-0.91 (m, 3H, $-\text{CH}_3$), δ 1.57-1.68 (m, 2H, CH_2), δ 2.37 (s, 3H, $-\text{CH}_3$), δ 4.08-4.16 (m, 2H, $-\text{OCH}_2$), δ 5.75 (d, $J = 48\text{ Hz}$, 1H, $-\text{CHF}$), δ 7.18-7.27 (m, 2H), δ 7.27-7.44 (m, 2H); ^{13}C -NMR (75 MHz, CDCl_3): δ 10.4, δ 19.4, δ 22.1, δ 29.9, δ 67.5, δ 87.2 (d, $J = 185\text{ Hz}$), δ 126.5, δ 131.1. ^{19}F -NMR (282 MHz, CDCl_3): δ -178.5 (d, $J = 48.7\text{ Hz}$). HRMS (EI+): exact mass calculated for $\text{C}_{12}\text{H}_{15}\text{FO}_2$ requires m/z 210.1056, found 210.1062. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μ m film) and the following separation program: 300 $^\circ\text{C}$ inlet, 300 $^\circ\text{C}$ detector, 100 $^\circ\text{C}$ oven, 2 $^\circ\text{C}/\text{min}$ gradient to 200 $^\circ\text{C}$, 50 $^\circ\text{C}/\text{min}$ gradient to 250 $^\circ\text{C}$, and 250 $^\circ\text{C}$ for 2 min. An

enantiomeric excess of 87% was estimated based on peak areas (see **Supplementary Figure 6**).



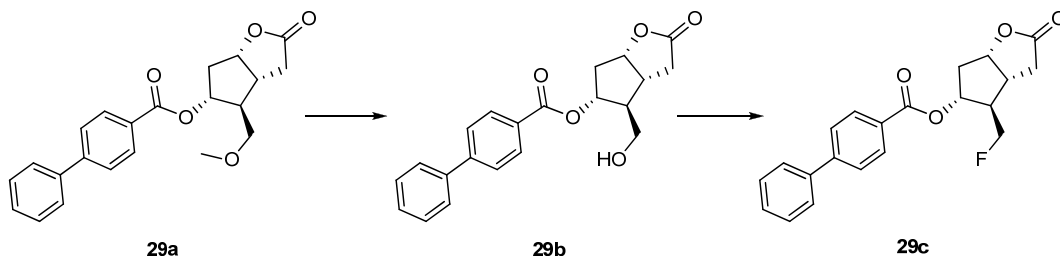
Conversion of 24 to 26. 103 mg (0.5 mmol) **24** were dissolved in 2 mL DMSO and added to 200 mL potassium phosphate buffer pH 8.0. The solution was added with var-H1 (final conc.: 2.5 μ M) and a cofactor regeneration system (final conc.: 500 μ M NADPH, 30 mM glucose-6-phosphate, 2 units/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 70 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (20% ethyl acetate: 80% hexane) to afford **25** (88 mg, 92%, colorless oil). ^1H -NMR (300 MHz, CDCl_3): δ 2.08 (s, 3H, CH_3), δ 3.65 (dt, $J=11.7, 3.6$ Hz, 1H), δ 3.85-3.92 (m, 2H), δ 5.34 (d, $J=7.8$ Hz, 1H), δ 7.26-7.40 (m, 5H). ^{13}C -NMR (75 MHz, CDCl_3): δ 14.22, δ 56.83, δ 63.18, δ 83.27, δ 125.99, δ 128.50, δ 129.12, δ 140.58, δ 166.60. HRMS (EI^+): exact mass calculated for $\text{C}_{11}\text{H}_{13}\text{NO}_2$ requires m/z 191.0946, found 191.0943. 88 mg (0.46 mmol) **25** and a catalytic amount (3 drops) of ethanol were dissolved in 3 mL dry dichloromethane under argon. The solution was placed in ice. After ten minutes, 66 μL DAST (0.46 mmol, 1 eq) were added to the mixture in aliquots of 22 μL every 30 minutes. The reaction was stirred in ice overnight. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by

(0.11 mmol, 1.5 eq) were added. The reaction was allowed to come to room temperature slowly (16 h) and then quenched by addition of 3 mL of saturated NaHCO₃ solution. The mixture was extracted with dichloromethane (4 x 15 mL), the organic phases were collected, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was purified by flash chromatography (20% ethyl acetate : 80% hexanes, then 33% ethyl acetate: 67% hexanes) to afford **27c** (9.4 mg, 66% yield, colorless oil). ¹H-NMR (300 MHz, CDCl₃): δ 2.21 (m, 2H), δ 2.52 (m, 1H), δ 2.81 (m, 2H), δ 3.30 (s, 3H), δ 3.78 (q, J= 7.2, 1H), δ 4.40 (ddd, J= 47.4, 24.6, 9.6 Hz, 1H, CHF), δ 4.41 (ddd, J= 47.4, 24.6, 9.6 Hz, 1H, CHF), δ 5.00 (m, 1H). ¹³C-NMR (75 MHz, CDCl₃): δ 27.05, δ 35.68, δ 36.85, δ 39.28, δ 52.98 (d, J= 18.3 Hz), δ 56.83, δ 83.18 (d, J= 139 Hz), δ 84.51, δ 179.29. ¹⁹F-NMR (282 MHz, CDCl₃): δ -225.8 (td, J= 48.2, 27.1 Hz). HRMS (EI⁺): exact mass calculated for C₉H₁₃FO₃ requires m/z 188.0849, found 188.0817.



Conversion of 28a to 28c. To a solution containing potassium phosphate buffer pH 8.0 and 1 mM **28a** in 1% DMSO was added with var-B3 (final conc.: 5 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 2 units/mL glucose-6-phosphate dehydrogenase). The reaction was stirred for 48 hours at room temperature, then worked-up as described above. The residue was purified by flash chromatography (25% ethyl acetate: 75% hexanes, then 33% ethyl acetate: 67% hexanes) to afford **28b** (75%, white powder). LC-MS (*m/z*): [M+H]⁺ 279.0. ¹H and ¹³C

NMR data were identical to those of the authentic standard. Two reaction vessels were prepared dissolving, under argon, 30 mg (0.11 mmol) **28b** in 3 mL dry dichloromethane in each vessel. 18.5 μ L DAST (0.13 mmol, 1.2 eq) were added each vessel in aliquots of 6 μ L aliquots every 30 minutes. The reaction was stirred at room temperature. After 4 hours, the reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was purified by flash chromatography (20% ethyl acetate : 80% hexane) to afford **28c** (33 mg, 55% yield, white powder). ^1H -NMR (300 MHz, CDCl_3): δ 2.50 (m, 4H), δ 2.96 (m, 2H), δ 4.54 (ddd, J = 47.4, 21.0, 9.6 Hz, 1H, CHF), δ 4.55 (ddd, J = 47.4, 21.0, 9.6 Hz, 1H, CHF), δ 5.12 (m, 1H), δ 5.41 (m, 1H), δ 7.44 (m, 2H), δ 7.56 (m, 1H), δ 8.01 (m, 2H). ^{13}C -NMR (75 MHz, CDCl_3): δ 36.20, δ 38.97, δ 40.30, δ 53.66 (d, J = 18.5 Hz), δ 73.16, δ 83.62 (d, J = 170 Hz), δ 84.92, δ 128.79, δ 129.89, δ 133.66, δ 166.22, δ 176.46. ^{19}F -NMR (282 MHz, CDCl_3): δ -226.7 (td, J = 48.0, 30.1 Hz). HRMS (EI^+): exact mass calculated for $\text{C}_{15}\text{H}_{16}\text{FO}_4$ requires m/z 279.1033, found 279.1041.



Conversion of 29a to 29c. To a solution containing potassium phosphate buffer pH 8.0 and 0.5 mM **29a** in 1% DMSO was added var-H1 (final conc.: 5 μ M), 1 mg/mL BSA, 1000 U/mL catalase, 4.1 U/mL SOD and a cofactor regeneration system (final conc.: 500 μ M NADPH, 30 mM glucose-6-phosphate, 2 units/mL glucose-6-phosphate

dehydrogenase). The reaction was stirred for 48 hours at room temperature, then worked-up as described above (e.g. compound 16). The residue was purified by flash chromatography (25% ethyl acetate: 75% hexanes, then 50% ethyl acetate: 50% hexanes) to afford **29b** (60%, colorless oil). LC-MS (m/z): $[M+H]^+$ 355.0. ^1H and ^{13}C NMR data were identical to those of the authentic standard. Two reaction vessels were prepared dissolving, under argon, 20 mg (0.05 mmol) **29b** in 2 mL dry dichloromethane in each vessel. 10 μL DAST (0.07 mmol, 1.2 eq) were added to each vessel in aliquots of 3 μL aliquots every 30 minutes. The reaction was stirred at room temperature. After 6 hours, the reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was purified by flash chromatography (20% ethyl acetate : 80% hexane) to afford **29c** (18 mg, 52% yield, white powder). ^1H -NMR (300 MHz, CDCl_3): δ 2.44 (m, 2H), δ 2.50-2.61 (m, 2H), δ 2.95-3.02 (m, 2H), δ 4.48 (ddd, J = 47.1, 21.3, 11.7 Hz, 1H, CHF), δ 4.64 (ddd, J = 47.1, 21.3, 11.7 Hz, 1H, CHF), δ 5.15 (m, 1H), δ 5.44 (m, 1H), δ 7.35-7.50 (m, 3H), δ 7.55-7.69 (m, 4H), δ 8.02-8.08 (m, 2H). ^{13}C -NMR (75 MHz, CDCl_3): δ 36.26, δ 39.00, δ 40.28 (d, J = 4.27 Hz), δ 53.76 (d, J = 18.2 Hz), 83.77 (d, J = 183.9 Hz), δ 84.81, δ 127.45 (2C), δ 127.52 (2C), δ 128.35, δ 128.45, δ 129.17 (2C), δ 130.44 (2C), δ 140.08, δ 146.35, δ 166.11, δ 176.55. ^{19}F -NMR (282 MHz, CDCl_3): δ -226.6 (td, J = 45.4, 30.2 Hz). HRMS (EI^+): exact mass calculated for $\text{C}_{21}\text{H}_{20}\text{FO}_4$ requires m/z 355.1346, found 355.1332.

P450 expression and purification. For the chemo-enzymatic transformations, P450 enzymes were used in purified form. Enzyme batches were prepared as follows. Two liters Terrific Broth medium were inoculated with an overnight culture (10 mL) of recombinant *E. coli* DH5 α cells harboring a pCWori plasmid encoding for the P450 variant under the control of Plac promoter. At OD₆₀₀=1.1, cultures were added with 0.5 mM IPTG and 0.5 mM δ -aminolevulinic acid (ALA) and transferred to 25°C. Cells were harvested after 24 hours. The cell pellet was resuspended in 25 mM TRIS (pH 8.0) and cell membranes were disrupted by sonication. Cell lysate was loaded onto a Q resin and the column was washed with 3 column volumes of 25 mM TRIS (pH 8.0), 150 mM NaCl. Bound protein was eluted with 25 mM TRIS (pH 8.0), 340 mM NaCl and concentrated using Millipore Centricon. After buffer exchange with 100 mM KPi (pH 8.0), protein samples were frozen and stored at -80 °C. Protein concentration was determined in duplicate from CO-difference spectra. Yields typically ranged between 100 and 500 mg protein per liter depending on the variant.

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